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U.S. DEPARTMENT OF ENERGY | Office of Science

LIFE SCIENCES DIVISION E-NEWSLETTER

September 30, 2008

In this issue:

DOE scientific focus area notes

Low Dose Radiation Research

Life Sciences Division Well Represented at Annual RSS Meeting

The Life Sciences Division was well represented at the Annual Meeting of the Radiation Research Society (RRS), held in Boston, September 20-24, 2008. Life Sciences Division researchers made eleven presentations, the authors and titles of which are listed here (presenters' names highlighted in bold):

Eleanor A. Blakely, Richard I. Schwarz, Kathleen A. Bjornstad, Chris J. Rosen, Polly Y. Chang, Derek Holman, Bahram Parvin, Al C. Thompson, Mina J. Bissell. DNA damage response and bystander effects of cells partially irradiated with 12.5 keV x-rays.

Sylvain Costes, James L. Chen, William Chou, Philippe Gascard, Mary Helen Barcellos-Hoff. Live cell imaging of radiation-induced 53BP1-GFP foci reveals active movement relative to chromatin

Amy Kronenberg, Stacey Gauny, Ely Kwoh, Cristian Dan, Michell Turker. Charged particle-induced autosomal mutations and the influence of the tissue microenvironment.

Sanchita Bhattacharya, Antoine Sniiders, Aris Polyzos, Sandhya Bhatnagar, Stephanie Chu, Francesco Marchetti, Xiu Lowe, **Andrew J. Wyrobek**. Differential gene transcript responses across tissues after whole body radiation in a mouse model.

Andrew J. Wyrobek, Antoine Snijders, Sanchita Bhattacharya, Sandhya Bhatnagar, Stephanie Chu, Aris Polyzos, Xiu Lowe, Francesco Marchetti. Molecular mechanisms of the radio-adaptive response for genomic damage in human and mouse tissues.

Faria Zafar, Sara B. Seidler, David Schild, **Claudia Wiese**. Homologous recombination contributes to the repair of double-strand breaks induced by HZE particles.

Francis A. Cucinotta, **Claudia Wiese**. Space radiation effects.

David H. Nguyen, Helen Oketch-Rabah, Jorge Reis-Filho, Daniel Medina, Mary Helen Barcellos-Hoff. Host irradiation and genotype affects characteristics of tumors arising from non-irradiated p53-null mammary epithelial transplants.

P. Kumari L. **Andarawewa**, Julien Deheuninck, William S. Chou, Sylvain Costes, Mary Helen Barcellos-Hoff. Persistent phenotypic responses induced by sparsely and densely ionizing radiation on human mammary epithelial cells.

Jenny Paupert, Mary Helen Barcellos-Hoff. Evaluation of irradiated fibroblasts on morphogenesis and breast cancer promotion using 3D co-culture and humanized stroma in vivo.

Mary H. Barcellos-Hoff, Christopher A. Maxwell, Markus C. Fleisch, Sylvain C. Costes, Shraddha A. Ravani, Bahram Parvin. Positive and negative non-targeted ionizing radiation effects that impact genomic instability in human epithelial cells.

Several researchers of the Life Sciences Division hold leadership positions in the society. **Amy Kronenberg** was recently elected as a new biology councilor; **Eleanor Blakely** chaired the meeting of the RRS History Committee; and **Andrew Wyrobek**, as president and representative of the Environmental

Mutagen Society (EMS), met with Drs. Peter Corry and Kathy Held, president and president elect of RRS to finalize a set of new agreements to enhance interaction opportunities between EMS and RRS member scientists. These agreements will grant reciprocity of member privileges at annual meetings and provide a framework for developing joint symposia and workshops on topics of mutual interest such as mechanisms of radiation mutagenesis and carcinogenesis and low-dose radiation risk assessment.
Andrew Wyrobek, 9/08

GTL-Genomics

Multi-channel Gel Electrophoresis and Continuous Fraction Collection Apparatus for High Throughput Protein Separation and Further Characterization

The main goal of Protein Complex Analysis Project (PCAP), a DOE funded GTL project, is to develop high throughput methods and pipelines to rapidly purify and identify the majority of stable protein complexes in a cell. Developments include methods employing various "tagging" strategies, 2-D Blue Native/SDS-PAGE analysis of membrane proteins and a novel "tagless" strategy to purify and identify majority stable protein complexes from a single large cell culture without using any genetic manipulation tools. Common in all of these is a need for a high throughput tool not only to isolate and purify proteins by gel electrophoresis but also to continuously elute protein bands into liquid fractions for further characterization, such as protein identification by mass spectrometry.

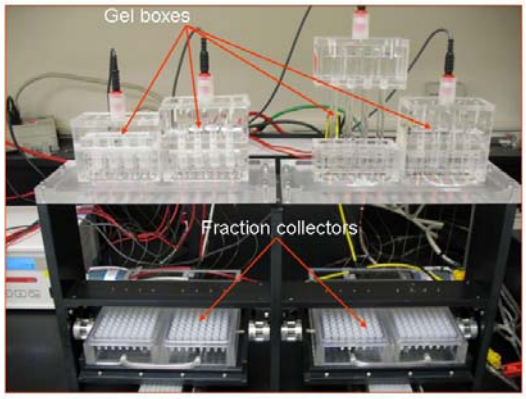

Fig-1. A multi-channel gel electrophoresis and continuous fraction collection instrument.

Such tool has now been developed jointly by **Jian Jin, Robert Nordmeyer** and **Earl Cornell** from the Engineering Division and bio-chemists **Ming Done**, **Megan Choi**, and PCAP project leader **Mark Biggin**. The multi-channel instrument (fig. 1) developed for the PCAP project is capable of continuous fraction collection as protein bands migrate off the bottom of gel columns. It was constructed based on a scheme that uses multiple short linear gel columns to achieve separation power similar to a long gradient gel, and a novel elution technique that allows continuous and simultaneous fraction collections of multiple channels. It has been demonstrated that fast and high resolution separation and fractionation of complex protein mixtures can be achieved on this system while running SDS-PAGE gels. In a 2.5-hour electrophoresis run, for example (fig. 2), each sample can be separated and eluted into 48 to 96 fractions over the mass range of ~10KD to 150KD; the sample recovery rate can reach 50% or higher; each channel can load up to 0.5mg material in 0.5 mL volume and a purified band typically elutes over 2~3 fractions (200 µl/fraction). Similar results could be obtained when running native gel electrophoresis on this instrument, but protein aggregation, mainly caused by sample over-loading and stacking, limited the loading capacity to about 50 ug per channel.

The traditional method for harvesting proteins is to cut or slice gels and re-dissolve them, which is tedious and low throughput. This technology provides an alternative, high throughput and more

convenient way of harvesting separated proteins for further processing and characterization, such as protein identification by the mass spectrometry.

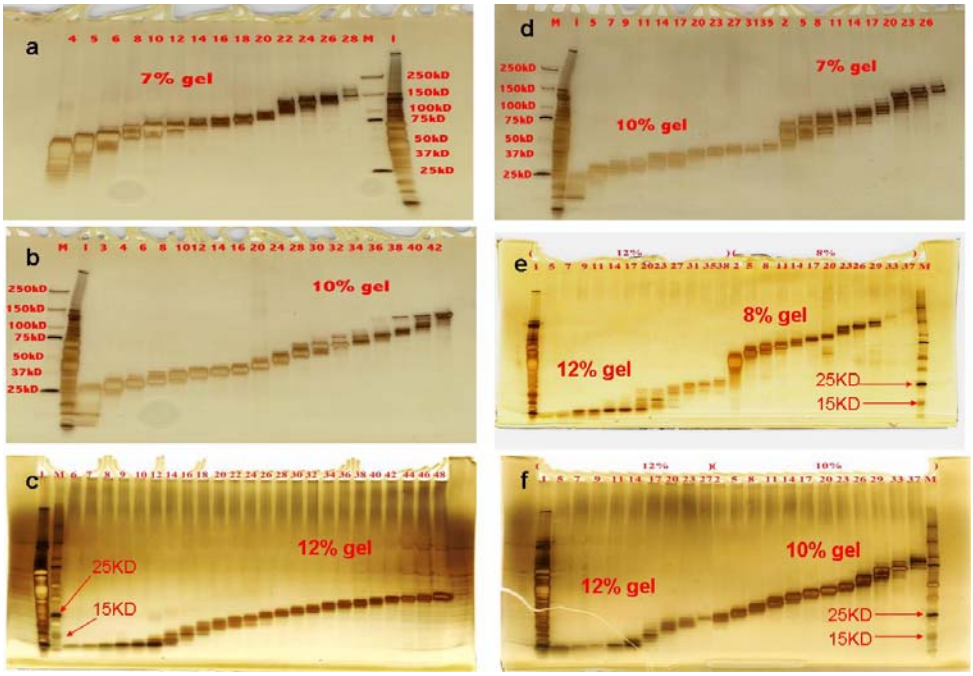

Fig-2. Elution of SDS-PAGE gels. Left panel (a, b, c): protein contents of collected fractions of crude extract of DvH using "direct" mode and different gel concentrations. Right panel (d, e, f): results obtained in a two-stage "stacking" mode and different gel combinations. The sample input lans are marked as "I", protein markers as "M" and other lanes labeled according to their fraction numbers.

Jian Jin, Kenneth Downing, 9/08

Nuclear Medicine

Planning for Bioenergy Future Research

Scientists in the Life Sciences Division are exploring how the nuclear tracers, imaging systems and data analysis they have developed for the real-time non-invasive imaging of biochemical processes in animals and humans can be used to study biochemical processes in plants. Objectives include systems for studying (1) drought-tolerant plants that can produce biofuels on land that is unsuitable for food production, (2) limiting factors in biofuel production, (3) the transport of contaminants through rock and soil, and (4) the sequestration of contaminants in plants. These topics will be discussed at a DOE BER Radiochemistry and Radionuclide Imaging Instrumentation Workshop to be held in Washington DC on November 4 and 5, 2008.

Stephen Derenzo, 9/08

Scientific news

The Structure of the Mre11 Protein Bound to DNA: First Glimpse of a Key DNA Repair Protein at Work
Repairing breaks in the two strands of the DNA double helix is critical for avoiding cancer. In humans and other organisms, a molecular machine called the MRN complex is responsible for finding and signaling double-strand breaks (DSBs), then launching the error-free method of DNA repair called homologous recombination.

In an article in the online October 3, 2008 issue of the journal Cell, **John Tainer** of the Life Sciences Division at the U.S. Department of Energy's Lawrence Berkeley National Laboratory, leading a team of his colleagues from the Scripps Research Institute, reveals how the central component of the MRN complex performs its essential functions. [Continue Press Release]
http://newscenter.lbl.gov/press-releases/2008/10/02/the-structure-of-the-mre11-protein-bound-to-dna/
Berkeley Lab News Center, Press Release, 10/2/08

Auer speaks at EBI Fall Biofuels Seminar Series
The Energy Biosciences Institute (EBI) has begun a new, biweekly seminar series this semester focusing on areas of importance within the biofuel field. The lectures take place every Tuesday at 4 p.m. in 116 Calvin Laboratory. The series starts September 9, 2008 with a talk by Berkeley Lab life scientist **Manfred Auer**, a microscopist working on imaging of plant cell walls. [More]
http://www.berkeley.edu/news/berkeleyan/2008/09/03_ebi.shtml
Today at Berkeley Lab, 9/8/08

Gray Attends Stand Up To Cancer Stand Up To Cancer Fundraiser
As a member of the AACR Board of Directors, **Joe Gray** attended the historic, nationally-televised, live program and after-party of Stand Up To Cancer (SU2C) at the Kodak Theatre in Los Angeles, California, on Friday, September 5, 2008. The AACR is the proud scientific partner for SU2C and applauds its leadership group for placing the spotlight on cancer research in more than 75 countries worldwide. The AACR provides scientific leadership, expert peer review, and grants administration to SU2C, an unprecedented collaboration among the major television networks, entertainment industry executives, celebrities, and prominent leaders in cancer research and patient advocacy.

The one-hour show was simultaneously broadcast live and commercial-free on ABC, CBS and NBC. The show included reports on cutting-edge translational research and performances by many legendary artists and celebrities. It was the highlight of an extensive public awareness campaign launched in May that also features a series of public service announcements in numerous media outlets and an interactive website that continues beyond September 5. All the funds donated by the public support the most promising translational research to bring advances in cancer treatment and prevention to patients

as quickly as possible. Berkeley Lab scientists have submitted several translational research proposals in response to the SU2C call.

[View the performance] http://www.standup2cancer.org/node/2734
[SU2C Press Release "Stand up to Cancer Announces More Than $100 Million Raised in Unprecedented Effort to End Cancer"] http://www.standup2cancer.org/node/2718
CG, 9/08

Life Sciences Scientific Division Holds Scientific Retreat
The Life Sciences Division held a Scientific Retreat on September 19, 2008 at the Joaquin Miller Community Center in Oakland, CA. The program committee, consisting of Life Sciences Postdoc Society Co-Presidents **Kelly Trego** and **Aris Polyzos** of the **Cooper** and **Marchetti** labs respectively, and **Mina Bissell**, created an exciting program that attracted many members' participation and lead to the success of this retreat.

A fundamental element of the program was the participation by postdocs and scientists in a lunchtime poster session, as well as in several 15 minute platform presentations. All postdocs and scientists were invited to submit a poster abstract; More than 50 researchers took advantage of the opportunity to share their work with the Division by presenting a poster. The goal was for the presentations to be accessible to the full breadth of scientists within the Division, since this would maximize everyone's appreciation of their colleagues' work and potentially kindle interesting new collaborations.


Francesco Marchetti presenting

Abstracts selected for talks featured both postdocs and scientists, were most lucid and compelling, and were chosen to reflect the diversity of research within the division. Most speakers were selected based on submitted abstracts but the program also featured invited speakers from the Division, UC Berkeley and Stanford University.

Keynote speaker Dr. Helen Blau of the Stanford University School of Medicine discussed the challenges faced when one studies adult muscle stem cells and how one might take advantage of live imaging techniques and bioengineering to surmount some of them. Turning then to bioengineered 3D culture substrata, Blau discussed her lab's recent efforts to identify molecules that regulate muscle stem cells.
CG, 9/08

Campisi Co-organizes Cold Spring Harbor Laboratory Conference on the Molecular Genetics of Aging
Life Scientist Judith Campisi (also of the Buck Institute) was one of the organizers of the Molecular Genetics of Aging Conference of Cold Spring Harbor laboratory, held September 24-28, 2008. The conference, also organized by Steven Austad (University of Texas San Antonio), David Sinclair (Harvard Med School), was first held in 1993 and has grown from a small meeting focused on a fledgling field to

an exciting venue for new and experienced investigators in a now fast-moving field. The conference brought together 250-300 scientists from the international community working on the genetics and molecular biology of aging. It provides an intense, in-depth forum for new findings and concepts in different areas of molecular aging research. 2008 Sessions included: (1) Genetics I; (2); Genomic stability (3); Mitochondria/metabolism (4) Cellular senescence/apoptosis/stress; (5) Stem cells; (6) Proliferative homeostasis; (7) Environment/interventions; and (8) Genetics II. Recently, remarkable progress has been made in establishing a molecular foundation in these areas, and their interrelationships are becoming increasingly clear. The conference featured anchoring talks by leading scientists working in these areas who chaired the sessions. A key strength of the series is that the majority of talks were selected from openly submitted abstracts three months prior to the meeting, providing ample opportunity for junior scientists to present their results and also for the presentation of important late-breaking findings. The conference, of which the next one is going to be held in 2010, fosters interactions among molecular gerontologists and biologists working in related areas, and provides a forum for developing new ideas and approaches to aging research.

Judith Campisi

CG, 9/08

Awards

Kronenberg Appointed CAMEE Member

Amy Kronenberg has been appointed as a member of the National Academies' Institute of Medicine (IOM) standing Committee on Aerospace Medicine and the Medicine of Extreme Environments (CAMMEE). She was one of four new appointees. The committee oversees NASA's life sciences research programs, it coordinates with NASA's Office of the Chief Health and Medical Officer to become informed of existing conditions and emerging issues related to medical care in space and define prospective activities (such as studies or analysis of medical needs and/or approaches to addressing those needs) to be conducted at the Institute of Medicine. The committee serves as a focal point for consideration of issues relating to the practice of medicine during space travel. The committee considers relevant scientific, technical, and policy issues including: the development of optimal aerospace medicine healthcare as an evolving multidisciplinary and international enterprise; health maintenance and care policies related to aerospace medicine; clinical research requirements and clinical strategies; and other relevant issues.

CG, 9/08

Recent publications (selected)

Fry RC, Svensson JP, Valiathan C, Wang E, Hogan BJ, **Bhattacharya S**, Bugni JM, Whittaker CA, Samson LD. Genomic predictors of interindividual differences in response to DNA damaging agents. *Genes and Development*, 2008 Sep 19. [Epub ahead of print] PMID: 18805990

> Human lymphoblastoid cells derived from different healthy individuals display considerable variation in their transcription profiles. Here we show that such variation in gene expression underlies interindividual susceptibility to DNA damaging agents. The results demonstrate the massive differences in sensitivity across a diverse cell line panel exposed to an alkylating agent. Computational models identified 48 genes with basal expression that predicts susceptibility with 94% accuracy. Modulating transcript levels for two member genes, MYH and C21ORF56, confirmed that their expression does indeed influence alkylation sensitivity. Many proteins encoded by these genes are interconnected in cellular networks related to human cancer and tumorigenesis.

Blagosklonny MV, **Campisi J**. Cancer and aging: more puzzles, more promises? *Cell Cycle,* 2008 Sep;7(17):2615-8. Epub 2008 Sep 16 PMID: 18719390

> In this issue of Cell Cycle, a new paper shows that metformin, an oral antidiabetic drug that activates AMP-activated protein kinase, prolongs both mean and maximal life span and prevents reproductive aging of female mice. Unexpectedly, metformin did not decrease the incidence of cancer in this mice strain. Here, we discuss the relationship between aging and cancer, the mechanism of metformin action, and the prospects of using this compound for life span extension in humans.

Chasis JA, Mohandas N. Erythroblastic islands: niches for erythropoiesis. *Blood*, 2008 Aug 1;112(3):470-8. PMID: 18650462

> Erythroblastic islands, the specialized niches in which erythroid precursors proliferate, differentiate, and enucleate, were first described 50 years ago by analysis of transmission electron micrographs of bone marrow. These hematopoietic subcompartments are composed of erythroblasts surrounding a central macrophage. A hiatus of several decades followed, during which the importance of erythroblastic islands remained unrecognized as erythroid progenitors were shown to possess an autonomous differentiation program with a capacity to complete terminal differentiation in vitro in the presence of erythropoietin but without macrophages. However, as the extent of proliferation, differentiation, and enucleation efficiency documented in vivo could not be recapitulated in vitro, a resurgence of interest in erythroid niches has emerged. We now have an increased molecular understanding of processes operating within erythroid niches, including cell-cell and cell-extracellular matrix adhesion, positive and negative regulatory feedback, and central macrophage function. These features of erythroblast islands represent important contributors to normal erythroid development, as well as altered erythropoiesis found in such diverse diseases as anemia of inflammation and chronic disease, myelodysplasia, thalassemia, and malarial anemia. Coupling of historical, current, and future insights will be essential to understand the tightly regulated production of red cells both in steady state and stress erythropoiesis

An X, Gauthier E, Zhang X, Guo X, Anstee DJ, Mohandas N, **Chasis JA**. Adhesive activity of Lu glycoproteins is regulated by interaction with spectrin. *Blood*, 2008 Sep 24. [Epub ahead of print] PMID: 18815288

The Lutheran (Lu) and Lu(v13) blood group glycoproteins function as receptors for extracellular matrix laminins. Lu and Lu(v13) are linked to the erythrocyte cytoskeleton through a direct interaction with spectrin. However, neither the molecular basis of the interaction nor its functional consequences have previously been delineated. In the present study, we defined the binding motifs of Lu and Lu(v13) on spectrin and identified a functional role for this interaction. We found that the cytoplasmic domains of both Lu and Lu(v13) bound to repeat 4 of the alpha spectrin chain. The interaction of full-length spectrin dimer to Lu and Lu(v13) was inhibited by repeat 4 of alpha spectrin. Further, resealing of this repeat peptide into erythrocytes led to weakened Lu-cytoskeleton interaction as demonstrated by increased detergent extractability of Lu. Importantly, disruption of the Lu-spectrin linkage was accompanied by enhanced cell adhesion to laminin. We conclude that the interaction of the Lu cytoplasmic tail with the cytoskeleton regulates its adhesive receptor function.

Weng KC, Noble CO, Papahadjopoulos-Sternberg B, **Chen FF**, Drummond DC, Kirpotin DB, Wang D, Hom YK, Hann B, Park JW. Targeted tumor cell internalization and imaging of multifunctional quantum dot-conjugated immunoliposomes in vitro and in vivo. *Nano Letters*, 2008 Sep;8(9):2851-7. Epub 2008 Aug 20. PMID: 18712930

Targeted drug delivery systems that combine imaging and therapeutic modalities in a single macromolecular construct may offer advantages in the development and application of nanomedicines. To incorporate the unique optical properties of luminescent quantum dots (QDs) into immunoliposomes for cancer diagnosis and treatment, we describe the synthesis, biophysical characterization, tumor cell-selective internalization, and anticancer drug delivery of QD-conjugated immunoliposome-based nanoparticles (QD-ILs). Pharmacokinetic properties and in vivo imaging capability of QD-ILs were also investigated. Freeze-fracture electron microscopy was used to visualize naked QDs, liposome controls, nontargeted QD-conjugated liposomes (QD-Ls), and QD-ILs. QD-ILs prepared by insertion of anti-HER2 scFv exhibited efficient receptor-mediated endocytosis in HER2-overexpressing SK-BR-3 and MCF-7/HER2 cells but not in control MCF-7 cells as analyzed by flow cytometry and confocal microscopy. In contrast, nontargeted QD-Ls showed minimal binding and uptake in these cells. Doxorubicin-loaded QD-ILs showed efficient anticancer activity, while no cytotoxicity was observed for QD-ILs without chemotherapeutic payload. In athymic mice, QD-ILs significantly prolonged circulation of QDs, exhibiting a plasma terminal half-life (t 1/2) of approximately 2.9 h as compared to free QDs with t 1/2 < 10 min. In MCF-7/HER2 xenograft models, localization of QD-ILs at tumor sites was confirmed by in vivo fluorescence imaging.

Raorane DA, Lim MD, **Chen FF**, Craik CS, Majumdar A. Quantitative and label-free technique for measuring protease activity and inhibition using a microfluidic cantilever array. *Nano Letters,* 2008 Sep 10;8(9):2968-2974. Epub 2008 Aug 23. PMID: 18720973

We report the use of a SiN x based gold coated microcantilever array to quantitatively measure the activity and inhibition of a model protease immobilized on its surface. Trypsin was covalently bound to the gold surface of the microcantilever using a synthetic spacer, and the remaining exposed silicon nitride surface was passivated with silanated polyethylene glycol. The nanoscale cantilever motions induced by trypsin during substrate turnover were quantitatively measured using an optical laser-deflection technique. These microcantilever deflections directly correlated with the degree of protease turnover of excess synthetic fibronectin substrate (K M = 0.58 x 10 (-6) M). Inhibition of surface-immobilized trypsin by soybean trypsin inhibitor (SBTI) was also observed using this system.

Bowman GR, Comolli LR, Zhu J, Eckart M, Koenig M, **Downing KH**, Moerner WE, Earnest T, Shapiro L. A polymeric protein anchors the chromosomal origin/ParB complex at a bacterial cell pole. *Cell*, 2008 Sep 19;134(6):945-55. PMID: 18805088

Bacterial replication origins move towards opposite ends of the cell during DNA segregation. We have identified a proline-rich polar protein, PopZ, required to anchor the separated Caulobacter crescentus chromosome origins at the cell poles, a function that is essential for maintaining chromosome organization and normal cell division. PopZ interacts directly with the ParB protein bound to specific DNA sequences near the replication origin. As the origin/ParB complex is being replicated and moved across the cell, PopZ accumulates at the cell pole and tethers the origin in place upon arrival. The polar accumulation of PopZ occurs by a diffusion/capture mechanism that requires the MreB cytoskeleton. High molecular weight oligomers of PopZ assemble in vitro into a filamentous network with trimer junctions, suggesting that the PopZ network and ParB-bound DNA interact in an adhesive complex, fixing the chromosome origin at the cell pole.

Beel AJ, Mobley CK, Kim HJ, Tian F, Hadziselimovic A, **Jap B**, Prestegard JH, Sanders CR. Structural studies of the transmembrane C-terminal domain of the amyloid precursor protein (APP): does APP function as a cholesterol sensor? *Biochemistry*, 2008 Sep 9;47(36):9428-46. Epub 2008 Aug 15. PMID: 18702528

The amyloid precursor protein (APP) is subject to alternative pathways of proteolytic processing, leading either to production of the amyloid-beta (Abeta) peptides or to non-amyloidogenic fragments. Here, we report the first structural study of C99, the 99-residue transmembrane C-terminal domain of APP liberated by beta-secretase cleavage. We also show that cholesterol, an agent that promotes the amyloidogenic pathway, specifically binds to this protein. C99 was purified into model membranes where it was observed to homodimerize. NMR data show that the transmembrane domain of C99 is an alpha-helix that is flanked on both sides by mostly disordered extramembrane domains, with two exceptions. First, there is a short extracellular surface-associated helix located just after the site of alpha-secretase cleavage that helps to organize the connecting loop to the transmembrane domain, which is known to be essential for Abeta production. Second, there is a surface-associated helix located at the cytosolic C-terminus, adjacent to the YENPTY motif that plays critical roles in APP trafficking and protein-protein interactions. Cholesterol was seen to participate in saturable interactions with C99 that are centered at the critical loop connecting the extracellular helix to the transmembrane domain. Binding of cholesterol to C99 and, most likely, to APP may be critical for the trafficking of these proteins to cholesterol-rich membrane domains, which leads to cleavage by beta- and gamma-secretase and resulting amyloid-beta production. It is proposed that APP may serve as a cellular cholesterol sensor that is linked to mechanisms for suppressing cellular cholesterol uptake.

Mukherjee B, Camacho CV, Tomimatsu N, **Miller J**, Burma S. Modulation of the DNA-damage response to HZE particles by shielding. *DNA Repair (Amst),* 2008 Oct 1;7(10):1717-30. Epub 2008 Aug 13. PMID: 18672098

Ions of high atomic number and energy (HZE particles) pose a significant cancer risk to astronauts on prolonged space missions. On Earth, similar ions are being used for targeted cancer therapy. The properties of these particles can be drastically altered during passage through spacecraft shielding, therapy beam modulators, or the human body. Here, we have used pertinent responses to DNA double-strand breaks (DSBs) to understand the consequences of energy loss versus nuclear fragmentation of Fe ions during passage through shielding or tissue-equivalent materials. Phosphorylation of histone H2AX and recruitment of 53BP1 were used to generate 3D reconstructions of DNA damage in human cells and to follow its repair. Human cells are unable to repair a significant portion of DNA damage induced by Fe ions. DNA-PK and ATM are required, to different extents, for the partial repair of Fe-induced DNA damage. Aluminum shielding has little effect on DNA damage or its repair, confirming that the hulls of the Space Shuttle and the International Space Station afford scant protection against these particles. Lead shielding, on the other hand, exacerbates the effects of Fe ions due to energy loss during particle

traversal. In sharp contrast, polyethylene (PE), a favored hydrogenous shield, results in DNA damage that is more amenable to repair presumably due to Fe-ion fragmentation. Human cells are indeed able to efficiently repair DSBs induced by chlorine ions and protons that represent fragmentation products of Fe. Interestingly, activation of the tumor suppressor p53 in Fe-irradiated cells is uniquely biphasic and culminates in the induction of high levels of p21 (Waf1/Cip1), p16 (INK4a) and senescence-associated beta-galactosidase activity. Surprisingly, these events occur even in the absence of ATM kinase implying that ATR may be a major responder to the complex DNA damage inflicted by Fe ions. Significantly, fragmentation of the Fe beam through PE attenuates these responses and this, in turn, results in better long-term survival in a colony-forming assay. Our results help us to understand the biological consequences of ion fragmentation through materials, whether in space or in the clinic, and provide us with a biological basis for the use of hydrogenous materials like PE as effective space shields.

Wang HW, Long S, Ciferri C, Westermann S, Drubin D, Barnes G, **Nogales E**. Architecture and flexibility of the yeast Ndc80 kinetochore complex. *Journal of Molecular Biology*, 2008 Sep 5. [Epub ahead of print]PMID: 18793650

Kinetochores mediate microtubule-chromosome attachment and ensure accurate segregation of sister chromatids. The highly conserved Ndc80 kinetochore complex makes direct contacts with the microtubule and is essential for spindle checkpoint signaling. It contains a long coiled-coil region with globular domains at each end involved in kinetochore localization and microtubule binding, respectively. We have directly visualized the architecture of the yeast Ndc80 complex and found a dramatic kink within the 560-A coiled-coil rod located about 160 A from the larger globular head. Comparison of our electron microscopy images to the structure of the human Ndc80 complex allowed us to position the kink proximal to the microtubule-binding end and to define the conformational range of the complex. The position of the kink coincides with a coil-coiled breaking region conserved across eukaryotes. We hypothesize that the kink in Ndc80 is essential for correct kinetochore geometry and could be part of a tension-sensing mechanism at the kinetochore.

Park CC, Rembert J, Chew K, Moore D, Kerlikowske K. High mammographic breast density is independent predictor of local but not distant recurrence after lumpectomy and radiotherapy for invasive breast cancer. *International Journal of Radiation Oncology, Biology, Physics*, 2008 Aug 7. [Epub ahead of print]PMID: 18692323

PURPOSE: Biologically meaningful predictors for locoregional recurrence (LRR) in patients undergoing breast-conserving surgery (BCS) and radiotherapy (RT) are lacking. Tissue components, including extracellular matrix, could confer resistance to ionizing radiation. Fibroglandular and extracellular matrix components of breast tissue relative to adipose tissue can be quantified by the mammographic breast density (MBD), the proportion of dense area relative to the total breast area on mammography. We hypothesized that the MBD might be a predictor of LRR after BCS and RT for invasive breast cancer. METHODS AND MATERIALS: We conducted a nested case-control study of 136 women with invasive breast cancer who had undergone BCS and RT and had had the MBD ascertained before, or at, diagnosis. Women with known recurrence were matched to women without recurrence by year of diagnosis. The median follow-up was 7.7 years. The percentage of MBD was measured using a computer-based threshold method. RESULTS: Patients with a high MBD (>/=75% density) vs. low (</=25%) were at increased risk of LRR (hazard ratio, 4.30; 95% confidence interval, 0.88-021.0; p = 0.071) but not distant recurrence. In addition, we found a complete inverse correlation between high MBD and obesity (body mass index, >/=30 kg/m(2)). In a multivariate Cox proportional hazards model, patients with MBD in the greatest quartile were at significantly greater risk of LRR (hazard ratio, 6.6; 95% confidence interval, 1.6-27.7; p = 0.01). Obesity without a high MBD also independently predicted for LRR (hazard ratio, 19.3; 95% confidence interval, 4.5-81.7; p < 0.001). CONCLUSION: The results of our study have shown that a high

MBD and obesity are significant independent predictors of LRR after BCS and RT for invasive breast cancer. Additional studies are warranted to validate these findings.

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Human cancer cells typically harbour multiple chromosomal aberrations, nucleotide substitutions and epigenetic modifications that drive malignant transformation. The Cancer Genome Atlas (TCGA) pilot project aims to assess the value of large-scale multi-dimensional analysis of these molecular characteristics in human cancer and to provide the data rapidly to the research community. Here we report the interim integrative analysis of DNA copy number, gene expression and DNA methylation aberrations in 206 glioblastomas-the most common type of adult brain cancer-and nucleotide sequence aberrations in 91 of the 206 glioblastomas. This analysis provides new insights into the roles of ERBB2, NF1 and TP53, uncovers frequent mutations of the phosphatidylinositol-3-OH kinase regulatory subunit gene PIK3R1, and provides a network view of the pathways altered in the development of glioblastoma.

Furthermore, integration of mutation, DNA methylation and clinical treatment data reveals a link between MGMT promoter methylation and a hypermutator phenotype consequent to mismatch repair deficiency in treated glioblastomas, an observation with potential clinical implications. Together, these findings establish the feasibility and power of TCGA, demonstrating that it can rapidly expand knowledge of the molecular basis of cancer.

Bloushtain-Qimron N, Yao J, Snyder EL, Shipitsin M, Campbell LL, Mani SA, Hu M, Chen H, Ustyansky V, Antosiewicz JE, Argani P, Halushka MK, Thomson JA, Pharoah P, Porgador A, Sukumar S, Parsons R, Richardson AL, **Stampfer MR**, Gelman RS, Nikolskaya T, Nikolsky Y, Polyak K. Cell type-specific DNA methylation patterns in the human breast. *Proceedings of National Academy of Sciences, U S A*, 2008 Sep 16;105(37):14076-81. Epub 2008 Sep 9. PMID: 18780791

Cellular identity and differentiation are determined by epigenetic programs. The characteristics of these programs in normal human mammary epithelium and their similarity to those in stem cells are unknown. To begin investigating these issues, we analyzed the DNA methylation and gene expression profiles of distinct subpopulations of mammary epithelial cells by using MSDK (methylation-specific digital karyotyping) and SAGE (serial analysis of gene expression). We identified discrete cell-type and differentiation state-specific DNA methylation and gene expression patterns that were maintained in a subset of breast carcinomas and correlated with clinically relevant tumor subtypes. CD44+ cells were the most hypomethylated and highly expressed several transcription factors with known stem cell function including HOXA10 and TCF3. Many of these genes were also hypomethylated in BMP4-treated compared with undifferentiated human embryonic stem (ES) cells that we analyzed by MSDK for comparison. Further highlighting the similarity of epigenetic programs of embryonic and mammary epithelial cells, genes highly expressed in CD44+ relative to more differentiated CD24+ cells were significantly enriched for Suz12 targets in ES cells. The expression of FOXC1, one of the transcription factors hypomethylated and highly expressed in CD44+ cells, induced a progenitor-like phenotype in differentiated mammary epithelial cells. These data suggest that epigenetically controlled transcription factors play a key role in regulating mammary epithelial cell phenotypes and imply similarities among epigenetic programs that define progenitor cell characteristics.

Fang Q, Noronha AM, Murphy SP, Wilds CJ, Tubbs JL, **Tainer JA**, Chowdhury G, Guengerich FP, Pegg AE. Repair of O(6)-G-Alkyl-O(6)-G interstrand cross-links by human O(6)-Alkylguanine-DNA Alkyltransferase. *Biochemistry*, 2008 Sep 20. [Epub ahead of print]PMID: 18803403

O (6)-Alkylguanine-DNA alkyltransferase (AGT) plays an important role by protecting cells from alkylating agents. This reduces the frequency of carcinogenesis and mutagenesis initiated by such agents, but AGT also provides a major resistance mechanism to some chemotherapeutic drugs. To improve our understanding of the AGT-mediated repair reaction and our understanding of the spectrum of repairable damage, we have studied the ability of AGT to repair interstrand cross-link DNA damage where the two DNA strands are joined via the guanine- O (6) in each strand. An oligodeoxyribonucleotide containing a heptane cross-link was repaired with initial formation of an AGT-oligo complex and further reaction of a second AGT molecule yielding a hAGT dimer and free oligo. However, an oligodeoxyribonucleotide with a butane cross-link was a very poor substrate for AGT-mediated repair, and only the first reaction that forms an AGT-oligo complex could be detected. Models of the reaction of these substrates in the AGT active site show that the DNA duplex is forced apart locally to repair the first guanine. This reaction is greatly hindered with the butane cross-link, which is mostly buried in the active site pocket and limited in conformational flexibility. This limitation also prevents the adoption of a conformation for the second reaction to repair the AGT-oligo complex. These results are consistent with the postulated mechanism of AGT repair that involves DNA binding and flipping of the substrate nucleotide and indicate that hAGT can repair some types of interstrand cross-link damage.

Syson K, Tomlinson C, Chapados BR, Sayers JR, **Tainer JA**, Williams NH, Grasby JA. Three metal ions participate in the reaction catalysed by T5 flap endonuclease. *Journal of Biological Chemistry*, 2008 Aug 11. [Epub ahead of print]PMID: 18697748

Protein nucleases and RNA enzymes depend on divalent metal ions to catalyse the rapid hydrolysis of phosphate diester linkages of nucleic acids during DNA replication, DNA repair, RNA processing and RNA degradation. These enzymes are widely proposed to catalyse phosphate diester hydrolysis using a "two-metal-ion mechanism". Yet, analyses of flap endonuclease (FEN) family members, which occur in all domains of life and act in DNA replication and repair, exemplify controversies regarding the classical "two-metal-ion mechanism" for phosphate diester hydrolysis. Whereas substrate-free structures of FENs identify two active-site metal ions, their typical separation of >4 A appears incompatible with this mechanism. To clarify the roles played by FEN metal ions, we report here a detailed evaluation of the magnesium ion response of T5FEN. Kinetic investigations reveal that overall the T5FEN catalysed reaction requires at least three magnesium ions, implying that an additional metal ion is bound. The presence of at least two ions bound with differing affinity is required to catalyse phosphate diester hydrolysis. Analysis of the inhibition of reactions by calcium ions is consistent with a requirement for two viable cofactors (Mg2+ or Mn2+). The apparent substrate association constant is maximised by binding two magnesium ions. This may reflect a metal dependent unpairing of duplex substrate required to position the scissile phosphate in contact with metal ion(s). The combined results suggest that T5FEN primarily uses a "two-metal-ion mechanism" for chemical catalysis, but that its overall metallobiochemistry is more complex and requires three ions.

Williams PT. Increases in weight and body size increase the odds for hypertension during 7 years of follow-up. *Obesity (Silver Spring),* 2008 Aug 28. [Epub ahead of print]PMID: 18756262

Changes in BMI and body size were compared to incident hypertension in 24,550 men and 10,111 women followed prospectively as part of the National Runners' Health Study to test whether long-term weight change affects hypertension risk. Incident hypertensions were reported by 2,143 men and 430 women during (mean +/- s.d.) 7.8 +/- 1.8 and 7.5 +/- 2.0 years of follow-up, respectively. Despite being active, men's and women's BMI increased 1.15 +/- 1.70 and 0.95 +/- 1.89 kg/m(2), respectively, and their waist circumferences increased 2.97 +/- 5.02 and 3.29 +/- 6.67 cm, respectively. Compared to those whose BMI declined, those who gained >/=2.4 kg/m(2) had an odds ratio (95% confidence interval) of 1.68 (1.45, 1.94) for becoming hypertensive if male and 1.42 (1.05, 1.92) if female. Men whose waist circumference increased >/=6 cm had an odds ratio of 1.22 (1.01, 1.47) for becoming hypertensive compared to those whose waists decreased. In both sexes, the odds for hypertension were significantly related to BMI at follow-up when adjusted for baseline BMI, but generally not to baseline BMI when adjusted for follow-up BMI. In the subset whose weights remained relatively unchanged during follow-up (+/-0.4 kg/m(2)), each kg/m(2) increment in BMI was associated with an odds ratio for becoming hypertensive of 1.19 (1.14, 1.24) in men and 1.11 (1.02, 1.20) in women. Thus, even among lean, physically active individuals: (i) weight gain increases hypertension risk; (ii) higher body weight increases the hypertension risk in a dose-dependent manner in the absence of any weight change; and (iii) there is no advantage carried forward to having been previously lean.Obesity (2008) doi:10.1038/oby.2008.396.

Williams PT. Effects of running distance and performance on incident benign prostatic hyperplasia. *Medicine and Science in Sports and Exercise*, 2008 Oct;40(10):1733-9. PMID: 18799982

PURPOSE: Benign prostatic hyperplasia (BPH) is generally not considered a preventable condition. Our goal is to assess whether running (a vigorous physical activity) and 10-km race performance (an indicator of cardiorespiratory fitness) reduce BPH risk. METHODS: Prospective cohort study of incident BPH in

28,612 nonsmoking, nonvegetarian, nondiabetic men. RESULTS: The 1899 men (6.64%) reported physician-diagnosed incident BPH during (mean +/- SD) 7.74 +/- 1.84 yr of follow-up. Survival analyses showed significantly lower risk with both longer distance run (km x wk(-1); P < 0.0001) and faster 10-km performance (m x s(-1); P = 0.0004) independent of age, BMI, and meat, fish, fruit, and alcohol intake. When adjusted for age, the fastest men (> or =4.0 m x s(-1)) had 32% lower risk than the slowest men (<3 m x s(-1); P = 0.0006). The decline in incidence extended throughout the performance range, with even the fastest category (> or =4 m x s(-1)) having significantly lower risk than the penultimate fastest category (3.5-4.0 m x s(-1); P = 0.03). The decline in BPH risk with running distance was independent of performance. BPH incidence was more strongly related to the average of the baseline and the follow-up distance run than to concurrent changes in running distance between baseline and follow-up. Incident BPH was significantly lower in men who ran >16 than <16 km x wk(-1) (P = 0.05), >32 than 16-32 km x wk(-1) (P = 0.02), and >48 than 32-48 km x wk(-1) (P = 0.04). CONCLUSIONS: Greater distances run per week may reduce BPH risk independent of BMI, 10-km performance, and diet. If the relationship is causal, then this health benefit accrues at greater exercise doses and intensities than the minimum guideline levels currently recommended.

Williams PT. Relationship of running intensity to hypertension, hypercholesterolemia, and diabetes. *Medicine and Science in Sports and Exercise*, 2008 Oct;40(10):1740-8. PMID: 18799983

PURPOSE: To estimate the independent relationships of running intensity with antihypertensive, LDL-cholesterol-lowering, and antidiabetic medication use when adjusted for running volume (km x d(-1)). METHODS: Self-reported medication use was compared cross-sectionally to running pace (m x s(-1) during usual run) in 25,552 male and 29,148 female National Runners' Health Study participants. RESULTS: The men ran a mean +/- SD of 5.2 +/- 3.1 km x d(-1) at 3.3 +/- 0.5 m x s(-1) (8.3 +/- 1.4 min x mile(-1)) and the women 4.7 +/- 2.9 km x wk(-1) at 3.0 +/- 0.4 m x s(-1) (9.2 +/- 1.8 min x mile(-1)). When adjusted for kilometers per day, each meter-per-second increment in intensity in men and women reduced the odds for antihypertensive drug use by 54% and 46%, respectively, reduced the odds for LDL-cholesterol-lowering medication use by 55% and 48%, respectively, and reduced the odds for antidiabetic medication use by 50% and 75%, respectively (all P < 0.0001). Compared with men who ran slower than 10 min x mile(-1), the odds for medication use in those who ran or exceeded a 7-min x mile(-1) pace were 72% less for antihypertensive, 78% less for LDL-cholesterol lowering, and 67% less for antidiabetic medications (the corresponding odds reductions in women were 61%, 64%, and 87%, respectively, for 8 min x mile(-1) or faster versus slower than 11 min x mile(-1)). Although usual running pace correlated significantly with a 10-km performance (male, r = 0.55; females, r = 0.49), usual pace remained significantly related to lower use of all three medications in men and antihypertension and antidiabetic medications in women when adjusted for a 10-km performance. CONCLUSIONS: Although these results do not prove causality, they show that exercise intensity is inversely associated with the prevalence of hypertension, hypercholesterolemia, and diabetes independent of exercise volume and cardiorespiratory fitness (10-km performance), suggesting that the more vigorous the exercise, the healthier the health benefits.